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An Experimental Study on the Behavior of Metastasized Regional Lymphnode after Removal of the Primary Tumor

by

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Content

I. INTRODUCTION

The greatest problem in cancer surgery has been that of metastasis, particularly lymphnode metastasis. Although it is a matter of fact that the more cancer cells appear in the blood stream from the original lesion, the higher the incidence of metastasis[19)66)69)], all these floating cells do not always develop to metastasis but most of these cells are destroyed without establishing metastasis, which has been obviously demonstrated by many investigations[52)65)]. A close relationship between occurrence of metastasis and host resistance is suggested particularly by the observation that the incidence of metastasis is markedly increased when host resistance is weakened by general irradiation, or administration of trypan-blue or cortisone[68)].

Clinically, surgeons are often confronted to early wide spread metastasis after seemingly radical operation. Such cases give an impression as if the removal of the primary lesion enhance vigorous growth of residual malignant cells[8)53)]. On the contrary, there

The gist of this article was reported at 20th Annual Meeting of Japanese Cancer Society and 16th Hokuriku Medical Assembly.

have been observations of amazingly long silent period after the removal of the primary lesion[16)18)54)] and sometimes clinical permanent cure is observed in cases of so-called palliative operation in which certain amount of tumor tissue is obviously left within the body[29)]. There are also some researchers who observed an inhibition[27)58)] or regression[45)] of residual cells after the removal of the primary tumor.

Lymphnode has been accepted to be invaded specifically by malignant tumors without exerting defence action, since lymphnode metastasis is so common and frequent in human malignancies[64)]. However, it is frequently observed at laparotomy that lymphnode swelling is strictly confined to the regional ones without metastasis in the distant lymphnodes. Such findings give an impression as if these regional lymphnodes are preventing wide spread of metastasis. BAENSCH[2)] observed regression and permanent cure in some cases in which irradiation was carried out upon primary tumor exclusive of metastasized regional lymphnodes, and he explained this healing process that the defence mechanism of residual lymphatic tissue came to surpass the activity of tumor cells owing to the stoppage of tumor cell supply to the lymphnodes.

Of course, there must be some occasions in which operative aggression such as removal of the primary tumor becomes useless or rather threatening at the time in which metastatic foci have shown a certain degree of development[8)]. However, if the primary tumor be removed prior to such stadium, there is a possibility that the host resistance is augumented enough to overcome the growth of tumor cells.

The present experiment was carried out in the aim of exploring and ascertaining this possibility.

II. MATERIALS AND METHOD

1. Experimental tumor and animal

Ascites hepatoma AH 130 following 458th generation of A1212 strain and 524th generation of A1363 strain and YOSHIDA sarcoma following 988th generation of No. 4378 strain and 1061st generation of No. 4575 strain from the SASAKI Institute were used.

Random bred female albino rats weighing 90 to 110 g, which are susceptible to above mentioned tumors, were fed by mixed diet and common water in the cages of metal and used for experiments.

2. Method of inoculation

Intraperitoneal 7 day growth of the tumor was aspirated by peritoneal tap and cell count was performed in a hemocytometer. Ascites tumor cell population was adjusted by saline to be approximately 1000×10^4 cells per 0.1 ml.

Inoculation was performed using tuberculin syringe and needle in the right foot. YOSHIDA sarcoma was implanted in the hypodermic space without injuring the tendon and the vessel at the midst of the footback, the needle being inserted between lst and 2nd fingers of the foot. Ascites hepatoma AH 130 was implanted in the muscle of the sole in the similar manner, since subcutaneous growth of ascites hepatoma often resulted in ulceration.

3. Operative procedure

Removal of the primary tumor was performed without anesthesia by amputation at

the distal end of the leg after ligature with silk-thread at the middle of the leg. Here, attention was paid not to leave tumor tissue. The proximal end of the amputation was covered with celloidin-alcohol. Amputation was performed under aseptic condition to avoid infection as possible. Bleeding, infection and local recurrence were not observed following this procedure.

4. Observation on growth of the metastasized lymphnodes

Size of the lymphnode observed by palpation was represented in the term of millimetre, which was transformed from the numbers of CHARRIÉRE's measure plate.

5. Histological examination

Removed lymphnodes were immediately fixed in a 10 per cent formalin and embedded in paraffin and cut logitudinally in the middle plane of the lymphnodes. At least 6 sections were prepared from a single lymphnode and they were stained doubly with hematoxylin and eosin.

Degree of metastasis was represented as follows ; when metastasis is not observed, ……(−), when tumor cells are found exclusively in peripheral sinus,……(+), when tumor cells are found to be invading from the peripheral sinus to medullary one,……(++), when the entire node is filled with tumor cells,……(+++). Histological reaction of the lymphnode was classified into 4 degrees of (−), (+), (++) and (+++). Here (−) means disappearance of germinal center and that of reticulum cells in the sinus, and (+++) hypertrophy and proliferation of these.

Ⅲ. LOCATION OF THE REGIONAL LYMPHNODE OF VARIOUS PARTS OF THE BODY

When lymphnode metastasis is concerned it is necessary to grasp the relationship between the area of lymphatic flow and the regional lymphnode. Location of the regional lymphnode of various parts of the body was studied following the method of JOB[24].

1. Method

Evans-blue of 0.1 ml of 0.5 per cent solution was subcutaneously injected in the foot and macroscopically stained lymphnodes were examined by autopsy with the lapse of time.

2. Results

Popliteal lymphnode of the same side was stained 1 hour after subcutaneous injection of Evans-blue solution in the footback, which spread to the lumbar and renal lymphnodes

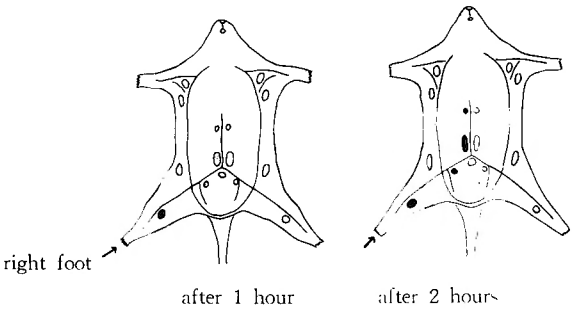

Fig. 1. Regional Lymphnodes of Various Parts of the Body Subcutaneous Injection of 0.5% Evans blue of 0.1 ml

2 hours after the injection as is shown in Fig. 1. The similar results was obtained when the solution was injected intramuscularly in the sole. In both occasion, lymphnodes of another side were never stained.

From this finding, the popliteal lymphnode was accepted to be the regional lymphnode of the foot. Based on the fact that metastasis in the popliteal lymphnode can be palpated easily without autopsy and in this occasion later growth can be studied, and furthermore from the reason that removal of the primary tumor can be performed with ease, implantation was carried out in the right foot and the right popliteal lymphnode was considered to be the primary regional one and the lumbar lymphnode to be the secondary regional node.

Ⅳ. RESULTS

A. Results of experiment in ascites hepatoma AH 130

1. Transplantation in the foot

Intramuscular inoculum in the right foot becomes macroscopically recognizable 5 days after the inoculation and gradually increases its size forming a tumor thereafter. All the animals whose tumor in the right foot reached a size of $8 \times 6 \times 7$ mm within 10 days showed continuous tumor growth and finally died of tumor. By the way, growth of the inoculum was always confined to the foot, without being accompanied by ulceration and without showing any tendency of spread to the leg. At the time of death, the popliteal lymphnode was markedly enlarged, as shown in Photo. 1, and lymphnodes in the root of the tail, in the lumbar region and renal lymphnodes were involved in a mass protruding from the retroperitoneum and compressing the rectum and bladder, whereas the accumulation of ascites was not observed as in intraperitoneal inoculation. Macroscopic metastasis to other organs was scarcely observed. The animals which showed susceptibility to the implanted tumor and regular development of tumor as mentioned in the above were used in the present experiment. Regular development of tumor was observed in 90.2 per cent in all the inoculations (175/194).

2. Time and pathway of lymphatic metastasis

At the various periods after inoculation in the foot, presence or absence of metastasis in the popliteal lymphnode and retroperitoneal lymphnodes was examined macroscopically and histologically by autopsy.

The results were represented in Tab. 1. Initial metastasis in the right popliteal lymphnode became recognizable later than 7th day of inoculation, and 10 days after the inoculation metastasis in this node was constantly observed in all experimental animals.

Tab. 1. Time of Popliteal Lymphnode Metastasis in Ascites Hepatoma AH 130

Day of Slaughter	No. of Animals	No. of Metastasis
5	3	0
7	8	3
9	10	9
10	10	10
12	5	5

Tab. 2. Spread of Lymphatic Metastasis in Ascites Hepatoma AH 130

Days after Inoculation	Popliteal Lymphnode				Lumbar Lymphnode				Renal Lymphnode				No. of Animals
	−	+	++	+++	−	+	++	+++	−	+	++	+++	
5	3	0	0	0	3	0	0	0	3	0	0	0	3
7	5	3	0	0	8	0	0	0	8	0	0	0	8
9	1	7	0	2	7	2	0	0	10	0	0	0	10
10	0	6	2	2	6	2	1	1	9	1	0	0	10
12	0	1	0	4	1	1	1	2	3	0	2	0	5

Behavior of metastasis in the right popliteal and retroperitoneal lymphnodes is summarized in Tab. 2. Metastasis was constantly observed in the right popliteal lymphnode and it was obviously observed that metastatic proliferation of tumor gradually spread to the lumbar lymphnodes and further to the renal nodes. In other words, it is presumed that the metastasis occurs firstly in the right popliteal lymphnode, then spreads to the lumbar and renal lymphnodes.

3. Results of primary tumor excision

Animals receiving no treatment and animals receiving foot amputation on the non-transplanted side 10 days after the inoculation were studied as control. In experimental group, amputation of the referred foot was performed 10 days after the inoculation at the stadium of constant metastasis in the right popliteal lymphnode and in another group of animals amputation was performed 13 days after the inoculation. In all these groups of animals, behavior of metastatic foci were studied. In experimental group, permanent cure following regression of metastasized regional lymphnodes was observed in some animals, and in some others metastatic tumor enlarged gradually and led animals to final death.

a. Rate of cure

As is shown in Tab. 3, no case of cure was observed both in non-treated animals and those of non-transplanted side amputation, and animals all died of tumor, whereas cure was observed in 9.5 per cent in the animals of 13th day amputation and in 25 per cent in those of 10th day amputation in experimental group, which is much higher compared with that of the former. In other words, regression of metastasized lymphnode, instead of progressive development, was observed in an unexpectedly high incidence, when the primary tumor was removed at an early stadium of metastasis in the regional lymphnode.

Tab. 3. Survival Time of Dead Animals and Rate of Cure in Ascites Hepatoma AH 130

	Average Survival Time	Rate of Cure	No. of Animals
Untreated Animals	17.3 (12 ~ 28)	0% (0/33)	33
Amputation on non-transplanted side	18.1 (14 ~ 27)	0% (0/14)	14
10th Day Amputation	24.9 (16 ~ 48)	25% (10/40)	40
13th Day Amputation	21.3 (15 ~ 30)	9.5% (2/21)	21

b. Survival time

Average survival time is represented in Tab. 3, i. e. 17.3 days in untreated animals,

18.1 days in animals of non-transplanted side amputation, while average survival time of animals died after removal of the primary tumor was 24.9 days in animals of 10th day amputation, the longest survival in this group being 48 days, and in animals of 13th day amputation, it was 21.1 days on an average. Survival rate curve also runs as in Fig. 2, which shows that in the animals of primary tumor excision prolongation of survival time is certainly observed, even if those animals finally died are concerned. Particularly, the earlier the time of operation, the larger is its effect.

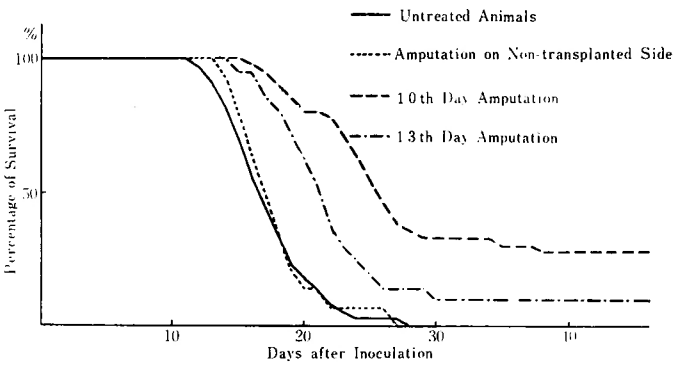

Fig. 2. Curve of Survival Rate in Ascites Hepatoma AH 130

4. Growth of metastasized regional lymphnodes

In both untreated animals and those of non-transplanted side amputation, approximately similar growth was observed as shown in Tab. 4. Size of the popliteal lymphnode was that of No. 5 to No. 12 of CHARRIÉRE's measure plate, No. 8.5 on the average, 10 days after the inoculation and rapidly enlarged thereafter until tumor death. Since there was no significant difference in survival time and growth of metastasized popliteal lymphnode between untreated animals and those of non-transplanted side amputation, it was assumed that such operative aggresion had no influence upon establishment of metastasis.

Tab. 4 Growth of Metastasized Popliteal Lymphnode in Control Animals in Ascites Hepatoma AH 130

Size of Popliteal Lymphnode in the Term of No. of CHARRIÉRE'S Measure Plate		Rat No.	Days after Inoculation																	
			5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Untreated Animals	A	60	4	5	5	6	10	12	13	14	†									
	A	11	4	4	4	5	6	7	10	13	15	18	19	†						
	A	66			5	5	6	8	10	14	19	23	26	30	32	†				
	A	69	3	3	4	5	5	5	7	9	13	16	19	21	24	†				
	A	13	5	6	7	7		9	11	12	12	13	14	16	18	20	24	28	30	†
Amputation on Non-transplanted side	A	58	3	3	5	6	6	8	10	14	15	19	†							
	A	81			4	6	7	7	11	12	13	13	14	24	†					
	A	67	3	3	4	5	5	5	7	9	13	16	20	22	26	34	†			
	A	70	3	3	3	4	6	8	11	13	16	22	26	28	32	35	†			
	A	2	3	5	7	7	8	9	9	12	12	14	15	18	20	25	30	30	35	†

Five animals were selected respectively from 33 untreated animals and 14 amputations on non-transplanted side.

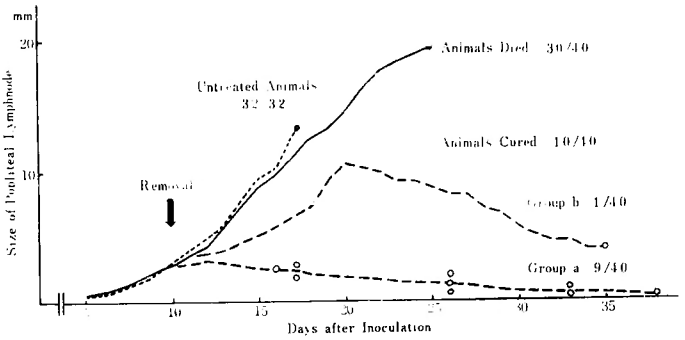

Fig. 3. Growth of Metastasized Regional Lymphnode in Ascites Hepatoma AH 130 (10th Day Amputation)

As is shown in Fig. 3, metastasized popliteal lymphnode in animals of 10th day amputation showed regression in 10 cases out of 40, and recurrence and death in 30 cases out of 40 after the removal of the primary tumor. Growth of the metastasized lymphnode in animals died finally showed roughly the same attitude as in untreated animals. However, average diameter of the popliteal lymphnode at the death was 18.2 mm, which was considerably larger than that of 12.2 mm in untreated animals. As is represented in Fig. 4, there was no correlation between the time of death and the size of the regional lymphnode. However, it may by accepted that the longer the survival time, the larger became the size of the popliteal lymphnode in both untreated animals and those of 10 day amputation.

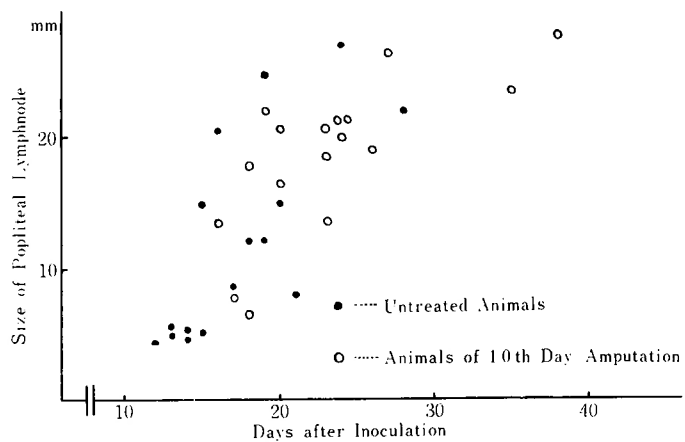

Fig. 4. Size of Metastasized Popliteal Lymphnode at the Time of Death in Ascites Hepatoma AH 130

As in Tab. 5, in animals of cure in metastatic tumor, growth of the metastasized lymphnode ceased after the removal of the primary tumor and gradually tended to regression, in 9 cases out of 40, as in Fig. 3, a, or the lymphnode enlarged slightly after the operation, however, followed by cessation of growth and tended to regression in 1 case out of 40, as in Fig. 3, b.

Tab. 5. Growth of Metastasized Popliteal Lymphnode in Animals Cured in Ascites Hepatoma AH 130

Size of Popliteal Lymphnode in the Term of No. of CHARRIÉRE's Measure Plate		Rat No.	Days after Inoculation																													
			5	7	9	11	13	15	17	19	21	23	25	27	29	31	33	35	38													
	10th Day Amputation	*A 91	3	3	4	6	6	6	8	8	6	6	**5**																			
		*A 92	3	4	6	7	7	8	9	8	8	7	7	**6**																		
		*A 44			8	8	9	9	9	8	8	7	7	**6**																		
		*A 16		4	4		5	6	7	7	9	10	11	11	8	7	7	6	6		**4**											
		A 18	4	5	6	6	7	7	8	7	7	6	6	5	5	4	4				**4**											
		*A 80	4	4	5	7	8	8	9	9	8		6	6	5	4		4	3	3	**3**											
		*A 81	4	4	6	7	8	9	9	10	10	9	8	7	6	6	5	4	4	4	3	3		2	2	0	0	0	**0**			
		A 21	3	4	5	6	8	8	8	7	7	6	5	5	5	4	4						3	3	3	2	2	2	**0**			
		A111	5	5	5	6	7	8	9	10	11	12	12	10	9	9	8	7	7	6	6	6						5			**3**	**0**
		*A 22		4	5	6	8	10	10	11	13	14	16	20	26	30	39	28	26	26	23	23	20	19	16	14	13	13	11	**11**		
	13th Day Amputation	*A 96	5	6	7	7	8	9	11	11	11	10	9	8	8	**6**																
		A 110	3	4	6	7	7	9	9	9	9	8	7				6						**6**									

*Cancer cells are found histologically at autopsy.

Behavior of metastasized popliteal lymphnode in animals of 13th day amputation showed, as in Fig. 5, also two different tendency of progressive growth to death in 19 cases out of 21 and cure in 2 cases out of 21. In the former, the metastasized lymphnode showed almost similar attitude as in untreated animals. Growth of the metastasized lymphnode in the latter was retarded in both of the cases and size of the popliteal lymphnode was No. 9 and No. 11 of CHARRIÉRE's measure plate, respectively, 13 days after the inoculation. The lymphnode further showed a tendency of cure, gradually regressing after the removal of the primary tumor. It is presumed that in these 2 cases the amputation was performed just at the stadium of initial metastasis, and in other cases of metastasis of (卅), all the animals died despite the operation.

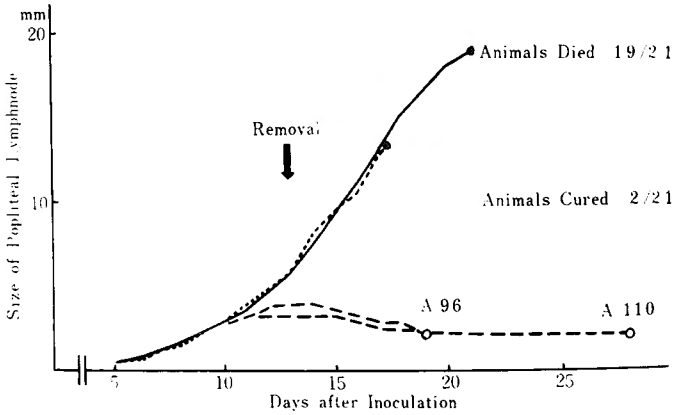

Fig. 5. Growth of Metastasized Regional Lymphnode in Ascites Hepatoma AH 130 (13th Day Amputation)

5. Histological findings of regional lymphnodes

Histological findings of tumor cell invasion in the lymphnode in untreated animals were those of circumscribed infiltration, namely invading into peripheral sinuses initially as is shown in Photo 2, then reaching medullary sinuses as is shown in Photo 3 and finally occupying the entire lymphnode as shown in Photo 4. Lymphatic reaction at initial metastasis was in the most part comprehended to be atrophy or disappearance of reticulum cells in sinus and germinal center as in Tab. 6, and as the metastasis developed, lymphatic reaction became hard to observe.

Histological findings of the popliteal lymphnode of animals died after removal of the primary tumor revealed the similar invasion of tumor cells as in untreated animals and lymphatic reaction was scarcely observed. On the contrary, among those which showed obvious regression of once metastasized lymphnode after removal of the primary tumor, residual tumor cells were histologically ascertained 6 to 28 days after the operation in 7 cases out of 40 in 10th day amputation and in 1 case out of 21 in 13th day amputation, revealing characteristic feature different from that of untreated animals.

In the animal A44, tumor cells situated in a mass in peripheral sinus, showing no particular change in tumor cells themselves 7 days after the operation as is shown in Photo 5 and 6. Furthermore, a zone chiefly consisted of reticulum cells slightly containing polynuclear leucocytes and lymphocytes was observed like a mole between lymphatic tissue and the mass of tumor cells. In the animal A22 sacrificed for histological study 25 days after the operation, the mass of tumor cells was surrounded by reticulum cells and these cells further intruding into the mass of malignant cells mingling together and karyorrhexis was also observed as shown in Photo 7 and 8. At the same time, a mass of reticulum cells was also observed here and there. The similar finding was also obtained in other animals of permanent cure. In other words, there appears a zone chiefly consisted of reticulum cells like a mole against the mass of tumor cells, which then surrounds the latter and intrudes into it to mingle together and finally tumor cells are completely replaced with the mass of the reticulum cells. Such a characteristic change around the mass of tumor cells was already observed conspicuously in the initial stadium in which no degeneration of tumor cells was observed. Lymphatic reaction of the entire node also differed from that of untreated animals, revealing hypertrophy and hyperplasia of the germinal center and hyperplasia of reticulum cells in sinuses as shown in Tab. 6 and Photo 9.

Among the cured cases in which residual tumor cells could not be found, approxima-

Tab. 6. Lymphatic Reaction in Ascites Hepatoma AH 130

	T. Z.	Germinal Center			Proliferation of Reticulum Cells			
		−	+	++	−	+	++	+++
Untreated Animals	+	62% (13/21)	38% (8/21)	0%	80% (17/21)	20% (4/21)	0%	0%
	++	90% (9/10)	10% (1/10)	0%	90% (9/10)	10% (1/10)	0%	0%
	+++	100% (10/10)	0%	0%	100% (10/10)	0%	0%	0%
Cured Animals	+~++	20% (2/10)	50% (5/10)	30% (3/10)	0%	30% (3/10)	30% (3/10)	40% (4/10)
	−	25% (1/4)	50% (2/4)	25% (1/4)	25% (1/4)	0%	50% (2/4)	25% (1/4)

tely the same lymphatic reaction was observed in 3 cases out of 40 in 10th day amputation and in 1 case out of 21 in 13th day amputation.

In 2 cases of 10th day amputation, which showed permanent cure despite metastasis in the lumbar lymphnode, the similar findings were observed in the lumbar lymphnode as in that of the poplitea.

B. Results of experiment in YOSHIDA sarcoma

1. Transplantation in the foot

Subcutaneous inoculum in the footback becomes recognizable macroscopically 3 days after the inoculation, which grows to a huge tumor thereafter and leads animals ultimately to death. Tumor development is usually confined to the foot and the leg is scarcely involved. At autopsy, the popliteal lymphnode is markedly enlarged as was seen in experiment with ascites hepatoma AH 130, the retroperitoneal lymphnodes jutting out in a mass from the retroperitoneum. Accumulation of ascites was scarcely observed and macroscopic metastatic foci were sometimes observed in the lung and kidney. Such regular development of tumor as mentioned in the above was observed in 94 per cent of all the inoculations (157/167).

2. Time and pathway of lymphatic metastasis

Appearance of metastasis in the popliteal and retroperitoneal lymphnodes after the inoculation in the foot was studied histologically at various periods. As shown in Tab. 7, popliteal lymphnode metastasis occurred earlier in YOSHIDA sarcoma and metastasis to this lymphnode was invariably observed 4 days after inoculation, revealing an amazingly rapid growth within the metastasized lymphnode. Spread to the popliteal and retroperitoneal lymphnode was quite in the similar manner as in ascites hepatoma AH 130, which is shown in Tab. 8. It was observed that metastasis first appeared in the popliteal lymphnode, then in the lumbar ones and finally invaded into the renal lymphnodes.

Tab. 7. Time of Popliteal Lymphnode Metastasis in YOSHIDA Sarcoma

Day of Slaughter	No. of Animals	No. of Metastasis
3	8	4
4	8	8
5	5	5

Tab. 8. Spread of Lymphatic Metastasis in YOSHIDA Sarcoma

Days after Inoculation	Popliteal Lymphnode				Lumbar Lymphnode				Renal·Lymphnode				No. of Animals
	−	+	++	+++	−	+	++	+++	−	+	++	+++	
3	4	3	1	0	8	0	0	0	8	0	0	0	8
4	0	4	2	2	3	2	2	1	7	1	0	0	8
5	0	0	1	4	0	1	2	2	4	0	1	0	5

3. Results of primary tumor excision

Animals receiving no treatment and those of 4th day non-transplanted side amputation were studied as control. For experimental animals, those receiving amputation at 4th day at which time invariable metastasis was observed in the popliteal lymphnode and

those of 6th day amputation were comparatively studied. Except those whose general condition was seriously impaired, all the animals were subjected to experiment. In this experiment also as in ascites hepatoma AH 130, there were animals of permanent cure and those of tumor death.

a. Rate of cure

Cure was not observed even in a case, as shown in Tab. 9, in untreated animals, in those of non-transplanted side amputation and in those of 6th day amputation, while in 18.8 per cent cure was observed in the animals of 4th day amputation.

Tab. 9. Survival Time of Dead Animals and Rate of Cure in YOSHIDA Sarcoma

	Average Survival Time	Rate of Cure	No. of Animals
Untreated Animals	11.2 (9~18)	0% (0/17)	17
Amputation on Non-transplanted Side	11.5 (9~15)	0% (0/10)	10
4th Day Amputation	15.0 (10~31)	18.8% (10/53)	53
6th Day Amputation	12.5 (9~21)	0% (0/16)	16

This is accepted to be a significantly high percentage, even if the rate of "no-take" of 6 per cent is taken into consideration. In short, in transplantation of YOSHIDA sarcoma also, some cases of cure, although few, can be observed, when the primary tumor is removed at an initial stage of regional lymphnode metastasis. However, when the rate of "no-take" was simultaneously considered, the frequency of cure was much less compared with that in ascites hepatoma AH 130.

b. Survival time

Average survival time was, as shown in Tab. 9, 11.2 days in untreated animals and 11.5 days in those of non-transplanted side amputation. On the other hand, average survival time of animals died after removal of the primary tumor was 15 days in animals of 4th day amputation, among which the longest survival time was 31 days. In animals of 6th day amputation, average survival time was 12.5 days. Curve of survival rate runs as in Fig. 6, suggesting a prolongation of survival time after removal of the primary tumor.

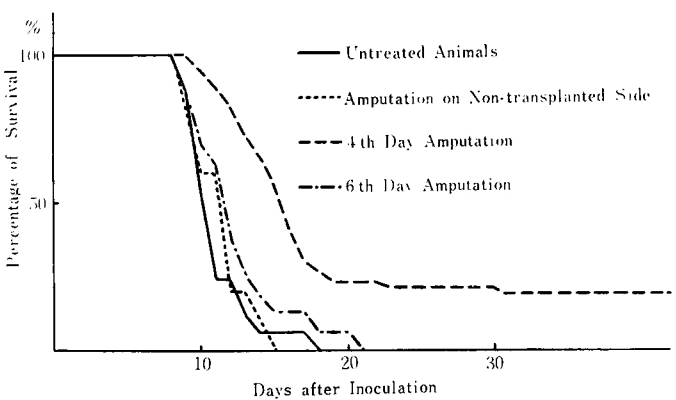

Fig. 6. Curve of Survival Rate in YOSHIDA Sarcoma

4. Growth of metastasized regional lymphnodes

Approximately similar development was observed in untreated animals, in those of non-transplanted side amputation and in those of 6th day amputation, as is summarized in Tab. 10.

Tab. 10. Growth of Metastasized Popliteal Lymphnode in YOSHIDA Sarcoma

Size of Popliteal Lymphnode in the Term of No. of CHARRIÈRE's Measure Plate

		Rat No.	Days after Inoculation											
			4	5	6	7	8	9	10	11	12	13	14	15
Untreated Animals	Y	92	4	5	7	10	13	24	†					
	Y	110	4	6	9	11	13	19	†					
	Y	222	3	5	8	13	13	15	18	†				
	Y	164	4	7	14	15	18	20	23	†				
	Y	228	3	4	7	11	14	19	21	24	32	†		
Amputation on Non-transplanted Side	Y	96	5	7	8	13	18	20	†					
	Y	122	3	5	7	13	15	18	22	†				
	Y	109	4	5	7	11	14	16	17	†				
	Y	113	4	6	9	14	17	20	23	†				
	Y	182	3	6	6	9	12	14	18	25	30	†		
6th Day Amputation	Y	118	4	6	7	12	17	20	22	†				
	Y	112	4	6	8	14	16	19	21	24	28	†		
	Y	126	3	5	8	13	13	15	17	18	20	†		
	Y	106	3	4	7	11	14	19	21	22	23	27	†	
	Y	224	5	7	8	10	11	15	20	24	27	30	34	†

Five animals were selected respectively from 17 untreated animals, 10 amputations on non-transplanted side and 16 of 6th day amputation.

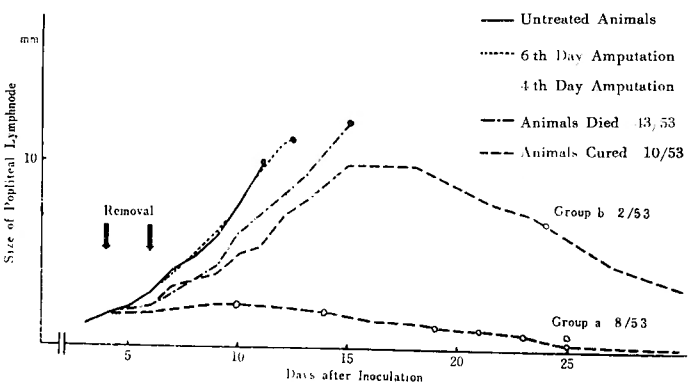

Fig. 7. Growth of Metastasized Regional Lymphnode in YOSHIDA Sarcoma

Behavior of metastasized popliteal lymphnode in animals of 4th day amputation was divided into 10 cured and 43 dead cases out of 53 respectively, as shown in Fig. 7. In animals ultimately died, growth was slightly retarded than in untreated animals, although appearance of development was nearly the same in both of these two. Average diameter of the popliteal lymphnode at the time of death was 10.7 mm, a little larger than that of 8.6 mm in untreated animals. This difference, however, was not so large as in ascites

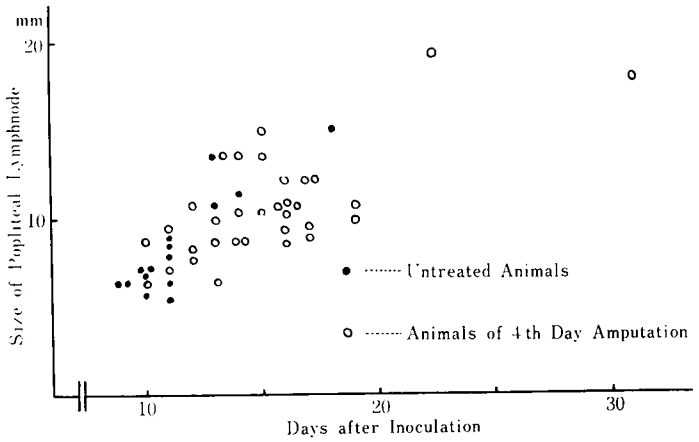

Fig. 8. Size of Metastasized Popliteal Lymphnode at the Time of Death in YOSHIDA Sarcoma

hepatoma AH 130, and the size itself also being smaller. These findings may be explained by shorter survival time in transplantation of YOSHIDA sarcoma. There was no correlation, as shown in Fig. 8, between the time of death and the size of the popliteal lymphnode. It may be, however, accepted that the longer the survival time, the larger becomes the size of the popliteal lymphnode in both untreated animals and those of 4th day amputation.

As is represented in Tab. 11, in animals of cure, metastasized lymphnode ceased its growth after removal of the primary tumor and gradually tended to cure in 8 cases out of 53 as in Fig. 7, a, or the lymphnode increased slightly its size after the operation, however, followed by cessation of growth sooner or later and finally tended to cure in 2 cases out of 53, as shown in Fig. 7, b.

Tab. 11. Growth of Metastasized Popliteal Lymphnode in Animals Cured in YOSHIDA Sarcoma.

Size of Popliteal Lymphnode in the Term of No. of CHARRIÈRE's Measure Plate

| 4th Day Amputation | Rat No. | Days after Inoculation | | |

5. Histological findings of regional lymphnodes

Tumor cell invasion in the lymphnode in untreated animals seemed to spread from the peripheral sinus to the medullary one as shown in Photo 10 and 11, being different from that of ascites hepatoma AH 130, the invasion progressed in a pattern of diffuse infiltration and proliferation of tumor cells also being rapid. Thus infiltrative tumor cells shortly occupy an entire lymphnode. Lymphatic reaction in initial metastasis was represented by atrophy or disappearance of the reticulum cells in the sinuses and germinal centers, as is shown Tab. 12.

Tab. 12. Lymphatic Reaction in YOSHIDA Sarcoma

		T. Z.	Germinal Center			Proliferation of Reticulum Cell			
			−	+	++	−	+	++	+++
Untreated Animals		+	82% (9/11)	18% (2/11)	0%	73% (8/11)	27% (3/11)	0%	0%
		++	100% (9/9)	0%	0%	89% (8/9)	0%	11% (1/9)	0%
		+++	100% (9/9)	0%	0%	100% (9/9)	0%	0%	0%
	Cured Animals	+~+++	60% (3/5)	0%	40% (2/5)	0%	60% (3/5)	40% (2/5)	0%
		−	20% (1/5)	80% (4/5)	0%	0%	80% (4/5)	20% (1/5)	0%

Residual tumor cells in the popliteal lymphnode of obvious regression were found in 5 cases out of 53 of 4th day amputation 6 to 58 days after the removal of primary tumor, histological findings of which revealing circumscribed infiltration of tumor cells as is shown in Photo 12, and in lymphatic reaction, hypertrophy and hyperplasia of germinal center and hyperplasia of reticulum cells in the sinuses were observed as shown in Tab. 12, being different also from that of untreated animals. Similar lymphatic reaction was also observed in the lymphnode in which tumor cells could not be found at histological examination, which resembled the results obtained in experiment with ascites hepatoma AH 130.

Histological findings of the popliteal lymphnode in 2 cases, in which metastatic tumor slightly enlarged after removal of the primary tumor, then ceased its growth and finally tended to cure, revealed that the entire lymphnode was filled with degenerated tumor cells containing necrosis in some area, and the proper structure of lymphnode was completely destructed. On the other side, no metastatic focus was found in the lumbar lymphnode and other organs.

V. DISCUSSION

One of the most important biological features of malignant tumor is an establishment of metastasis. As is already stated by OTA[44] and ZEIDMAN[69], in establishment of metastasis following factors are indispensable, that is, proliferation of tumor in original site, floating of tumor cells in blood or lymphatic stream, leaving from original tumor cell mass, settlement of tumor cells reaching distant organs and tissues through these vessels, and proliferation of settled tumor cells to become metastatic foci. There have been many attempts to investigate mechanism of metastasis by reproducing such metastasis experimen-

tally. Although there have been numerous experimental studies which pursued influence of hematogenous scattering of tumor cells on establishment of metastasis, very few experimental reports are seen concerning metastasis through lymphatic pathway[30)34)50)70)]. In addition, most of these, if any, are concerned with study of lymphnode by injecting tumor cells into the lymph vessels[34)50)70)], and has a disadvantage that this maneuver are largely different from the natural process of spontaneous metastasis, which rather resembles implantation.

The author of the present paper succeeded in producing lymphnode metastasis constantly by implanting ascites hepatoma AH 130 and YOSHIDA sarcoma in the foot of rats, which was devised in the aim of reproducing lymphnode metastasis experimentally in a condition resembling spontaneous metastasis as possible. By this procedure, inoculum grew in the site of implantation, at the same time producing lymphnode metastasis in the poplitea. Metastatic growth of tumor cells further spread to the lumbar and renal lymphnodes and finally led animals to death. Histological picture of initial metastasis was proliferation of tumor cells in peripheral sinus, which gradually developed to intrude into medullary one and the entire lymphnode was replaced with tumor cells finally.

This finding is accepted to resemble so closely to lymphnode metastasis in human cancer[60)72)].

There is no report of obsrevation on the course of metastasis in an individual animals, and SCHATTEN[53)], DRUCKREY[8)], and ZEIDMAN[21)] also made observation of metastasis by autopsy in their experiments. It must be more justifiable to conceive that the fluctuation of growth rate in an individual organism is caused by the alteration of host resistance than to conceive that it is due to that of malignancy itself[17)56)] In this respect it is assumed to be significant in studying the change in host resistance that the author of the present experiment could pursue the destiny of metastatic focus in an individual animals by palpation of the popliteal lymphnode.

At the surgery of malignant tumor, wide spread recurrence or metastasis is frequently observed, which sometimes gives an impression as if the removal of the primary tumor resulted to enhance growth of residual tumor cells[8)53)]. As an influencing factor upon metastasis, survival time of the animals should be considered together with operative aggression, as was pointed out by ROUSSY[48)].

As promoting factors to metastasis, which are included in operative aggression, draining[48)] of tumor cells into the blood and lymph vessels around the tumor, massage[28)33)] of the tumor and incision[9)41)] of it are considered topically, and general influences such as anesthesia[15)], bleeding[59)] and increase in cortisone[46)68)] due to stress reaction have been reported to affect the establishment of metastasis. LEWIS[31)] also postulated that operative aggression increases the incidence of metastasis by reducing host resistance. Accordingly, these factors brought about by operative procedure should be avoided as possible. Based on the result of the present experiment using foot implantation in which approximately similar survival time and growth of metastasized lymphnode were observed in both untreated animals and those of non-transplanted side amputation, it must be concluded that operative aggression brought about by the procedure in the present experiment has little influence upon establishment of metastasis.

Concerning the influence of survival time on metastasis, DRUCKREY[8] observed in his experiment using Flexner-Jobling carcinoma that dissemination and death resulted earlier in the animals of removal of the primary tumor than in those without the operation. Hence, he considered the operative aggression itself as an influencing factor upon metastasis and concluded that it is difficult to predict prolongation of survival time after surgery. BESERGA[3] pointed out that the incidence of metastasis showed a tendency of increase as time elapsed longer after removal of the primary tumor. SATO[49] and others observed in his experiment of tail implantation that retroperitonal lymphnode metastasis was more frequent and its growth also was more conspicuous in animals of primary tumor excision than in those without the removal, and he explained that this difference is due to longer observation in the former until death or slaughter, in other words this was due to the fact that longer interval of time was permitted for tumor growth.

In the results of the present experiment, the longer the survival time, the larger was the size of regional lymphnode at the time of death in both animals operated on and those of control. It is accepted quite natural that the longer the survival time, the more frequent the incidence of metastasis should become.

As was mentioned in the above, although there are some researchers who insist an occurrence or increase in incidence of metastasis after surgery, sometimes extremely long incubation time is observed between removal of primary tumor and clinical manifestation of metastasis, as was reported by GUMMEL[18], GINSBERG[16] and SCHMIDT[54].

PETERSEN[45] and KUNITOKI[29] observed clinical permanent cure in cases of so-called palliative operation in which some amount of tumor tissue was left. At times, regression of metastatic tumor is observed after removal of the primary tumor as reported by MILLER[37].

These observations make it difficult to conceive that surgery always enhances growth of tumor.

At duplicate implantation of experimental tumor, MATSUYAMA[35] observed that incidence of "take" was lower at the second implantation than at the initial one, and he explained that lower incidence of "take" at the 2nd implantation was caused by an establishment of inhibitory factor within the organism which was induced by tumor growth of initial implantation. SUGIMOTO[58] observed unexpectedly low frequency of recurrence of residual tumor tissue after palliative removal of mouse tumor, and attributed the cause to improvement of general condition due to removal of the most part of the tumor. KAGETSU[26] also pointed out based on his similar experiment that such healing mechanism was improved by activation of the reticuloendothelial system and put an emphasis on a close relationship between general condition and the reticuloendothelial function. OLD[42] reported that there exists a state of reticuloendothelial hyperfunction during the course of experimental tumor as determined by phagocytic activity for colloidal carbon, and the phagocytic activity decreases as the general condition becomes worse with the enlargement of tumor. We[40] also reported that such reticuloendothelial hyperfunction lasts in the case of regression of experimental tumor. It is also reported that congo-red index is improved in patients of stomach cancer when the tumor is favorably removed[71].

As is obvious from these reports and observations, it is presumed that some general

factor, which hinders or inhibits growth of tumor, appears during the course of tumor development in tumor transplantation, in which mechanism reticuloendothelial function largely participates.

Accordingly, it is presumed that there exists some antagonism between potentiality of tumor growth and host resistance.

SUGANO[57] observed, in his experiment of tail implantation of ascites hepatoma MH 134, some cases of cure after amputation of the tail performed at a stadium of establishment of obvious metastasis in the retroperitoneal lymphnodes.

In the present experiment also, permanent cure following regression of metastasized lymphnode was observed in considerable frequency by the removal of the primary tumor in an extremely early stadium of metastasis. As to the histological appearance of lymphnode which showed obvious regression after removal of the primary tumor, reticulum cells were chiefly observed appearing around the mass of tumor cells like a mole, then intruding into the mass and finally the mass being replaced with these reticulum cells. Such change was markedly observed already in the stadium in which degeneration of tumor cells was hardly observed. As lymphatic reaction, hypertrophy and hyperplasia of germinal center and hyperplasia of reticulum cells in the sinuses were observed.

Nevertheless, wide spread metastasis and extremely low rate of cure are experienced in clinical cases of seemingly early and adequately radical operation. This must be partly because of operative aggression. However, much more important reason must consist in the fact, as KALLENBACH[25] observed residual metastatic foci in one third or one half of all the cases in his statistic study of autopsy in cancer patient within 6 weeks after operation, that most of clinically early operation cannot always be accepted as histologically early operation[14]. On the contrary, it is almost impossible to assume that completely radical operation could be performed without remaining any tumor cells microscopically in all cases of 5 year survivals after operation of stomach cancer. In other words, it is presumed from the present experiment that regression of tumor cells occurred in the clinical cases of operation performed at an appropriate period, with resulting favorable long survivals of more than 30 per cent.

Although there is a concept of WALTHER[64] that the lymphnode is a suitable soil for cancer growth, implantation of tumor cells into the lymphnode is more difficult than into other organs[50], and WATERMAN[67] reported an amazing lytic activity of lymphnode extract on tumor cells. ZEIDMAN[70], KUSCHFELD[30], FLAKS[11] and SCHMIDT[55] ascertained experimentally destructive picture of tumor cells within the regional lymphnode, and concluded that lymphnode has an inhibitory effect on malignant tumor growth. Moreover, AULER[1] asserted that the existence of lymphnode is definitely important in animals to prevent establishment of metastasis, based on his experimental demonstration in Flexner-Jobling carcinoma that manifestation of metastasis was earlier and more conspicuous in implantation in animals whose lymphnode had been previously removed than in control animals. On the other hand, there are some reports of clinical cases suggesting the existence of an antitumoral activity of the regional lymphnode[22][60][61].

Concerning the significance of stromal reaction in tumor, there are some investigators who regard it as a mere secondary reaction against destructive products of tumor paren-

chyma[12)21)32)]. On the contrary, some researchers such as IMAI[20)], BÖHMIG[5)] and others[38)39)43)47)72)] regard this reaction as a defence reaction of organism against tumor. SAITO[51)] insisted that the stromal reaction somewhat varies depending upon the structure of surrounding tissues around the tumor. Among these cells participating in stromal reaction, there are lymphocyte, plasma cell, neutrophil, eosinophil, mast cell and histiocyte.

Concerning the lymphnode particularly, FROMME[13)] stated that lymphnode is a filter of lymphatic spread of tumor and the mesenchymal tissue in the lymphnode which is principally consisted of reticulum cells exerts an active defence reaction against tumor cells and their metabolic products, as it exerts in bacterial infections. Furthermore, BLACK[4)] reported that prognosis was favorable in those cases in which sinus histiocytosis modified by marked hyperplasia of reticulum cells in the sinuses and increase in number of germinal center were observed in non-metastasized regional lymphnode. TAKIZAWA[62)] observed in autopsy study that there was atrophy or disappearance of sinus endothelium in the lymphnode in which, even if partially, tumor cells invaded vividly, while in the lymphnode in which degenerative change of invasive tumor cells was found, hyperplasia of sinus endothelium was conspicuous. From this observation, he presumed that the function of sinus endothelium which belongs to the reticuloendothelial system has particular significance against proliferation of tumor cells in the lymphnode.

In the present experiment also, lymphnode exerted defence reaction against tumor, and it is presumed that the function of cells which belong to the reticuloendothelial system has significance against proliferation of tumor cells in the lymphnode.

It was not, however, clarified whether this defence reaction is the reaction as the regional lymphnode or a sign of general defence mechanism.

As we have reported previously, we have obtained some findings suggesting an increase in general host resistance from the observations on distant lymphnodes, from changes in serum and from fluctuation of general reticuloendothelial function[40)]. Based on these findings, it is reasonably presumed that the reaction of the regional lymphnode against tumor is not only due to reaction derived from the local lymphnode, but also due to general antitumoral mechanism of organism. In other words, the fact that regression occurred in the metastasized lymphnode after removal of the primary tumor is interpreted that defence reaction of organism against tumor came to surpass the growth of tumor by the major removal of tumor and finally came to inhibit metastatic growth, revealing a reaction chiefly consisted of reticulum cells proliferation in the regional lymphnodes.

Although there was some difference in the rate of cure after removal of the primary tumor depending on the time of operation, survival time of animals finally died was longer than in those without operation. This finding suggests that removal of the primary tumor can possibly prolong the survival time, when the operative aggression is controlled within the extent not to impair the host resistance, even if considerably advanced metastasized lymphnode is remained within the organism, and this can be interpreted to suggest the importance of performance of operation. On the other side, lower rate of cure in YOSHIDA sarcoma compared with that in ascites hepatoma AH 130 demonstrates important factors of malignancy of tumor itself.

Lately, a concept of extended radical operation is asserted by DENCK[7)] and JINNAI[23)].

McWHIRTER[36] observed in breast cancer that more favorable results were obtained by irradiation after simple mastectomy compared with that after radical mastectomy. CRILE[6] also insisted that clinical results were worse in prophylactic removal of normal lymphnode than in occasion in which the lymphnode was removed after tumor invasion and the results were not always fatal even if metastasized lymphnodes were remained. GUMMEL[18] and DUNPHY[10] asserted that complete removal of the regional lymphnode should be performed at a certain period of time after removal of the primary tumor, so that these lymphnodes may capture tumor cells scattered at operation. In animal experiment, TOKUYAMA[63] obtained worse results in extended-radical operation than in palliative one. Furthermore, judging from the results of the present experiment also, it is questionable, when lymphnode function is deliberately considered, to perform extended radical operation in which large amount of lymphnodes simply swollen are removed.

VI. SUMMARY

Ascites hepatoma AH 130 and YOSHIDA sarcoma were inoculated in the foot of rats and the primary tumor was removed at a stage of regional lymphnode metastasis. Behavior of the regional metastasized lymphnodes was followed up and the results obtained are summarized as follows ;

1. Popliteal lymphnode metastasis was constantly observed 10 days and 4 days after inoculation of ascites hepatoma AH 130 and YOSHIDA sarcoma, respectively.

2. No cure was observed in untreated animals, while cure was observed in 25 per cent of 10th day amputation and in 9.5 per cent of 13th day amputation in ascites hepatoma AH 130, and in 18.8 per cent of 4th day amputation in YOSHIDA sarcoma.

3. Average survival time was 17.3 days in untreated animals and 18.1 days in animals of non-transplanted side amputation, and average survival time of animals died after removal of primary tumor was 24 9 days in 10th day amputation and 21.1 days in 13th day amputation in the experiment of ascites hepatoma AH 130.

In the experiment of YOSHIDA sarcoma, average survival time was 11.2 days in untreated animals and 11.5 days in animals of non-transplanted side amputation, and average survival time of animals died after operation was 15 days in 4th day amputation and 12.5 days in 6th day amputation. The earlier the time of operation, the longer was the survival time.

4. Behavior of growth in the popliteal lymphnode was almost similar in both untreated animals and those died after removal of the primary tumor. In animals which showed tendency of cure, two different courses were observed in the behavior of metastasized lymphnode, that is, one revealing cessation of tumor growth after surgery and gradual regression and another revealing transient enlargement after surgery, which, however, ceased sooner or later and finally tended to cure.

5. Average diameter of metastasized popliteal lymphnode at the time of death was 12.2 mm in untreated animals and 18.2 mm in 10 th day amputation in ascites hepatoma AH 130, and 8.6 mm in untreated animals and 10.7 mm in 4th day amputation in YOSHIDA sarcoma. Here it is assumed that average diameter of metastasized popliteal lymphnode at the time of death was larger in experimental group, because there was some growth

brought about by prolongation of survival time after removal of the primary tumor.

6. At histological studies in animals died, invasion of tumor cell is easily permitted without any reaction, showing atrophy or disappearance of reticulum cells in the sinuses and germinal center and shortly the entire lymphnode comes to be filled with tumor cells in animals finally died. On the contrary, in animals tended to cure, mass of tumor cells was surrounded by reticulum cells, which then intruded into the mass and finally tumor cells became hard to be found. Furthermore, as the lymphatic reaction, hypertrophy and hyperplasia of germinal center and hyperplasia of reticulum cells in the sinuses were also observed.

As has been mentioned in the above, if the primary tumor is removed at an early stage of regional lymphnode metastasis, there sometimes occurs regression of metastasized lymphnode without further proliferation. This is interpreted that defence mechanism of organism against tumor comes to surpass the growth potentiality of tumor owing to the removal of the primary tumor and it comes to inhibit the growth of metastatic tumor, revealing a histological finding of reaction in the regional lymphnode principally consisted of reticulum cells.

Accomplishing the present experiment, I gratefully express my hearty gratitude to Prof. Dr. ICHIO HONJO for his kind guidances and inspiring encouragement throughout the experiment, and I am also debted to Mr. HATANO, Pathological Department in the University, and Dr. MIZUMOTO and the members of our clinic for their kind and friendly advices and helps.

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(* in Japanese)

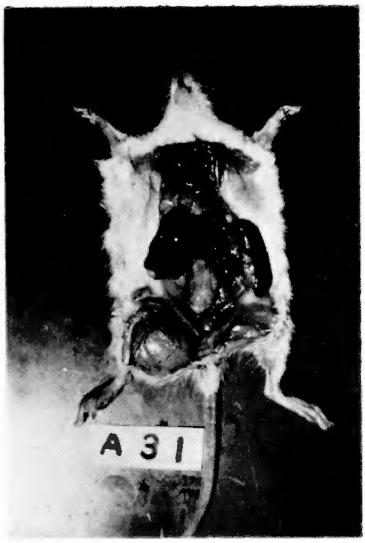

Photo. 1. Macroscopic finding of popliteal and lumbar metastasis at autopsy (AH 130)

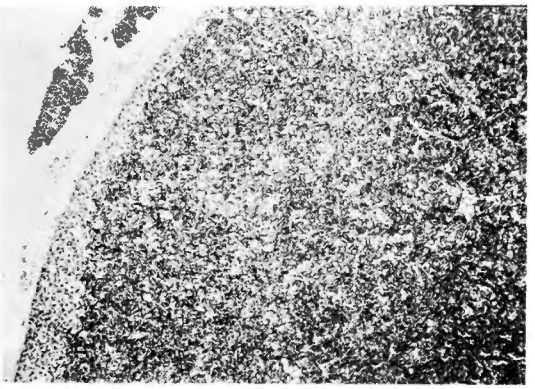

Photo. 2 Histological appearance of metastasized popliteal lymphnode in control group (AH 130) Metastasis (+) (×100 H. E.)

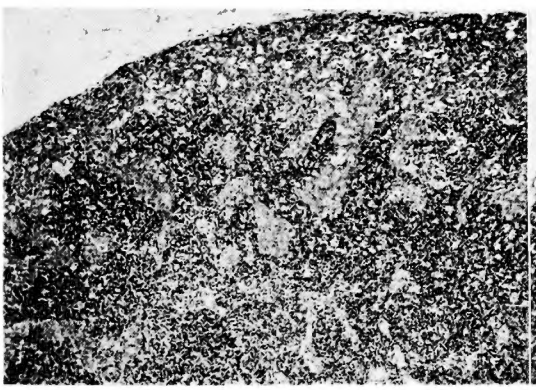

Photo. 3. Histological appearance of metastasized popliteal lymphnode in control group (AH 130) Metastasis (++) (×100 H. E.)

Photo. 4. Histological appearance of metastasized popliteal lymphnode in control group (AH 130) Metastasis (+++) (×100 H. E.)

Photo. 5. Histological appearance of metastasized popliteal lymphnode in group of regression (AH 130) (×150 H. E.)

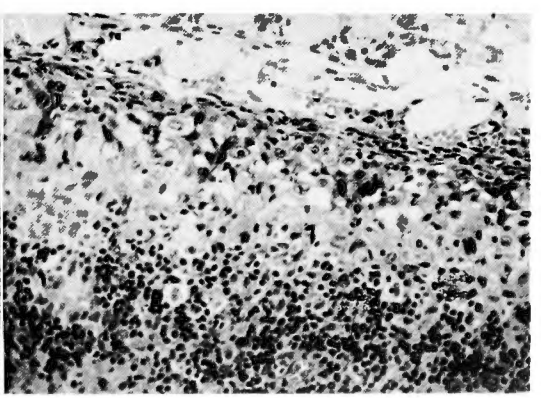

Photo. 6. Histological appearance of metastasized popliteal lymphnode in group of regression (AH 130) (×300 H. E.)

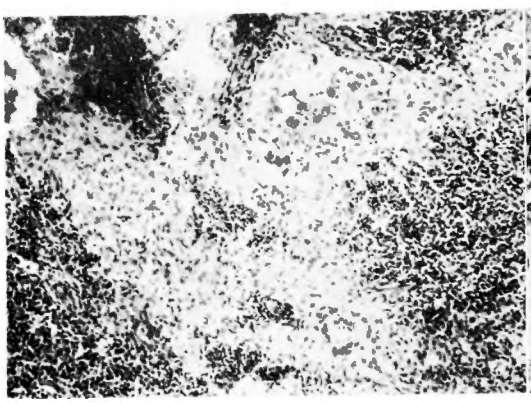

Photo. 7. Histological appearance of metastasized popliteal lymphnode in group of regression (AH 130) (×150 H. E.)

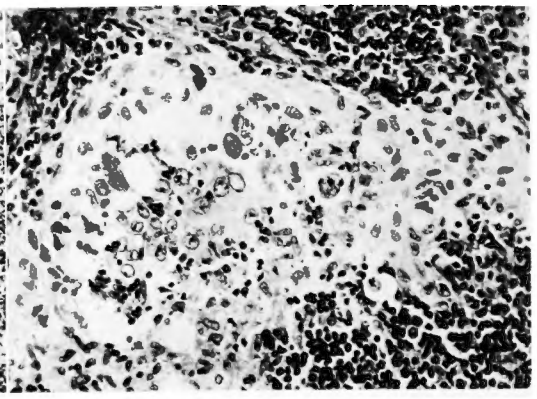

Photo. 8. Histological appearance of metastasized popliteal lymphnode in group of regression (AH 130) (×300 H. E.)

Photo. 9. Histological appearance of metastasized popliteal lymphnode in group of regression (AH 130) (×50 H. E.)

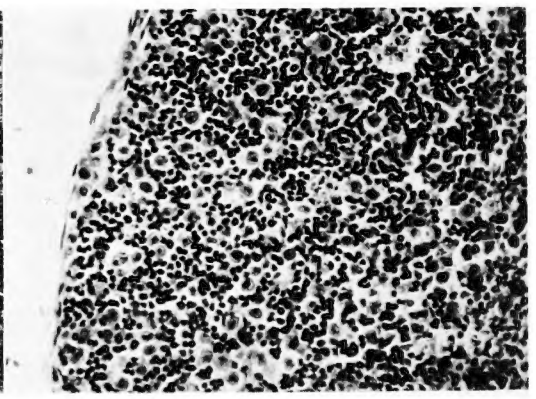

Photo. 10. Histological appearance of metastasized popliteal lymphnode in control group (YOSHIDA sarcoma) (×380 H. E.)

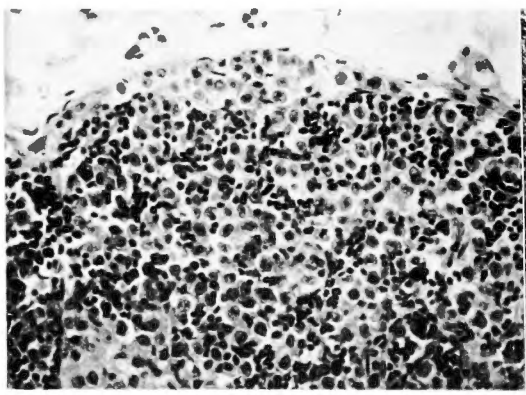

Photo. 11. Histological appearance of metastasized popliteal lymphnode in control group (YOSHIDA sarcoma) (×380 H. E.)

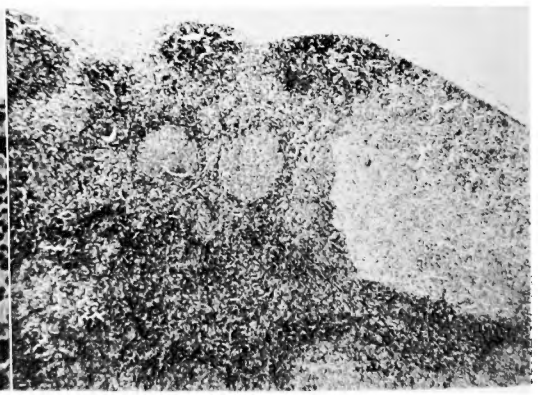

Photo. 12. Histological appearance of metastasized popliteal lymphnode in group of regression (YOSHIDA sarcoma) (×50 H. E.)

和 文 抄 録

主腫瘍剔除後の領域転移リンパ節の態度（実験的研究）

金沢大学医学部第2外科学教室（指導：本庄一夫教授）

崎　田　重　康

癌手術に当つて最も問題となるのは癌転移，殊にリンパ節転移の問題である．充分に根治手術が施行出来たと思われる症例に，早期の広汎な再発転移を経験することがある．しかるにその反面，明らかに腫瘍組織の一部が残留された，いわゆる姑息的切除例に極めて永い潜伏期を経過したり，臨床上の永久治癒と判断される例を認めることがある．

そこで癌の原発巣の剔除が転移リンパ節に及ぼす影響を検するために，腹水肝癌AH130，吉田肉腫をラッテの足部に移植し，膝リンパ節転移の現われた時期に主腫瘍剔除を行ない，以後の膝リンパ節の動向を検索して次の結果を得た．尚非切除対照群を無処置群とし，対照としては又偽手術群として健足切断群ももうけた．更に主腫瘍切除群を実験群とした．

1．治癒率は無処置群には1例も認められなかつたが，実験群中AH 130の10日目切断群25%，13日目切断群9.5%，吉田肉腫の4日目切断群18.8%に認められた．

2．平均生存日数は，AH 130では無処置群17.3日，健足切断群18.1日で，実験群中の死亡群の平均生存日数は10日目切断群24.9日，13日目切断群21.1日であり，吉田肉腫では無処置群11.2日，健足切断群11.5日，実験群中の死亡群の平均生存日数は4日目切断群15日，6日目切断群12.5日であつて，手術時期が早期な程延命効果も大である．

3．膝リンパ節の発育態度は，無処置群及び実験群中死亡せるものはほゞ同様な態度を示し，治癒群では術後発育が停止し次第に縮少するものと，術後尚やゝ増大するが早晩発育は停止して遂に治癒するに至るものとがある．

4．死亡群の膝リンパ節の平均直径は，AH 130の無処置群12.2㎜，10日目切断群18.2㎜，吉田肉腫の無処置群8.6㎜，4日目切断群10.7㎜で実験群の方が大きいのは主腫瘍剔除による生存日数の延長のため残存転移巣の発育があつて大きくなつたものと考えられる．

5．組織学的所見では，死亡群ではなんらの反応をみることなしに癌細胞の侵入をうけ，洞内細網細胞及び反応中枢の萎縮乃至消失を来たし，まもなくリンパ節は癌細胞で充満された状態となるが，治癒群では癌細胞の集団を細網細胞が取り囲み，更にその内に侵入し，ついには癌細胞は認め難くなる．またリンパ節の反応像に於いても反応中枢の肥大増生及び洞内細網細胞の増生を認めた．

上述の如く領域リンパ節転移のごく初期に主腫瘍を剔除すると，転移リンパ節は増殖を続けないでregressionする場合がある．これは原発巣の剔除により，生体の癌に対する防衛反応が上廻つて転移巣に抑制的に働き，組織学的には領域リンパ節に細網細胞を主体とした反応が認められたものと解される．

本論文の要旨は第20回日本癌学会総会，第16回北陸医学会総会に於いて講演発表した．